Quick Start Guide

For Research Use Only. Not for use in diagnostic procedures.

Agilent OnePGT Solution

An NGS-based, single workflow solution for PGT-M, PGT-SR and PGT-A

Agilent OnePGT Solution is a genome-wide, next-generation sequencing (NGS)-based system designed to integrate pre-implantation genetic testing (PGT) for monogenic disorders (PGT-M), translocations (PGT-SR), and aneuploidies (PGT-A) in a single workflow. Agilent OnePGT Solution includes the REPLI-g Single Cell Kit for whole genome amplification, the Agilent OnePGT Library Prep Kit for the generation of NGS-ready libraries, and the Agilent Alissa OnePGT Software for data analysis and reporting.

Specifications

	PGT-M for monogenic disorders	PGT-SR for translocations	PGT-A for aneuploidies
Reference family members required?	Yes (see table on page 3)	No	No
Pre-test for feasibility	Optional	NA	NA
Illumina platform[1] sequencing set up	2 x 150 bp ≥16M paired-end reads per library	2 x 75 bp ≥1.3M paired-end reads per library[2]	2 x 75 bp ≥1.3M paired-end reads per library[2]

1 Supported instruments include NextSeq 500/550 and HiSeq 2500 (rapid run mode).

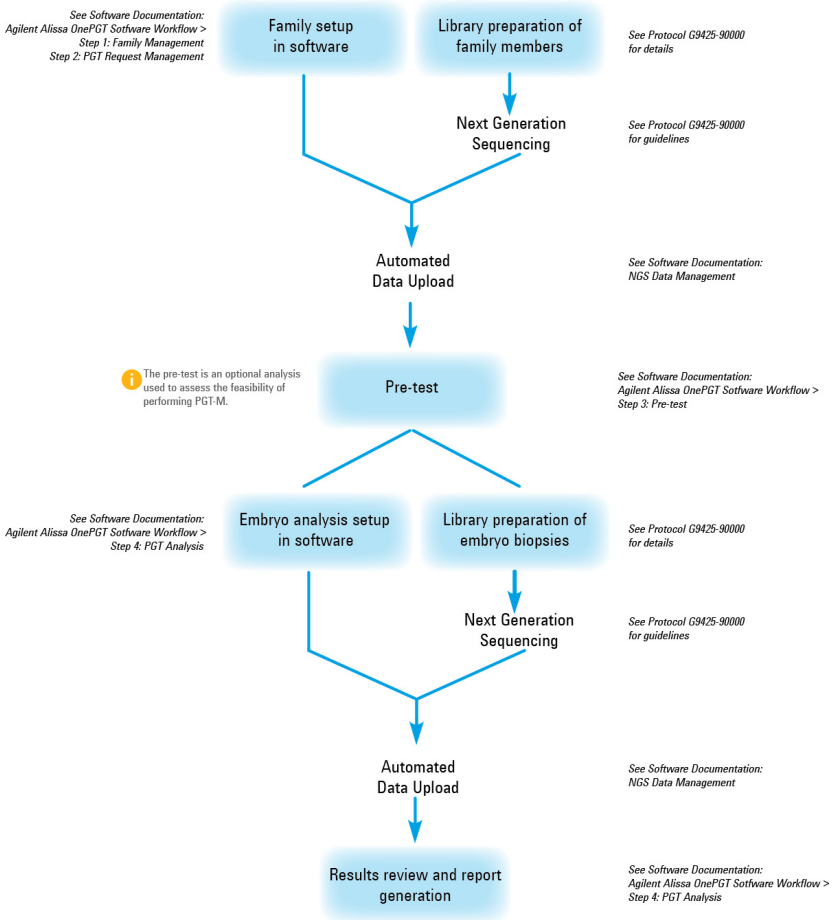
2 PGT-SR and PGT-A samples can also be sequenced together with PGT-M samples using the recommended PGT-M sequencing set up.

Limitations

- Only human blastomere and trophectoderm biopsies which have been whole genome amplified using the REPLI-g Single Cell kit, according to the instructions provided in the Agilent OnePGT Library Prep Kit Protocol, are valid input samples for the Agilent OnePGT Solution.
- The OnePGT Solution is not intended to detect haploidy, polyploidy, or mosaicism.
- No automated calls can be made for monogenic disorder loci located within 2 Mb from the start/end of the chromosome for blastomere biopsies, or within 1 Mb for trophectoderm biopsies.
- Only reciprocal translocations, including Robertsonian translocations, are supported.
- Translocations smaller than 2 Mb are not supported. The optimal translocation size is ≥10 Mb. For translocations between 2 Mb and 10 Mb, the chance of obtaining an inconclusive result is higher.
- Translocations between homologous chromosomes or involving the Y-chromosome are not supported.
- Analysis of consanguineous families is not recommended.
- The limit of detection for PGT-A is 20 Mb for blastomere and 5 Mb for trophectoderm biopsies.

PGT-M for monogenic disorders

Workflow



Pre-test

The pre-test is an optional analysis that can be performed on the gDNA samples from the parents and reference family members, to assess the feasibility of performing a PGT-M analysis with this family setup before processing the embryo biopsies. Even when choosing not to perform a pre-test upfront of embryo analysis, the results of the pre-test can be found in the final report. For more information, refer to the Agilent Alissa OnePGT Software documentation section *Step 3: Pre-test*. The information from the pre-test, as well as the sequencing data from the parents and reference family members, can be reused when additional IVF cycles are performed within 5 years.

Reference family members

In addition to the whole genome amplified embryo biopsy, PGT-M analysis also requires unamplified gDNA from the parents and one or more reference family members. The unamplified reference gDNA is extracted from blood and processed using the same OnePGT library preparation protocol. The table below shows which family members can be used for analysis depending on the inheritance mode of the monogenic disorder. The family member nomenclature is based upon the relationship with the test-embryo. For more information, refer to the software documentation section *Creating a PGT-M Request*.

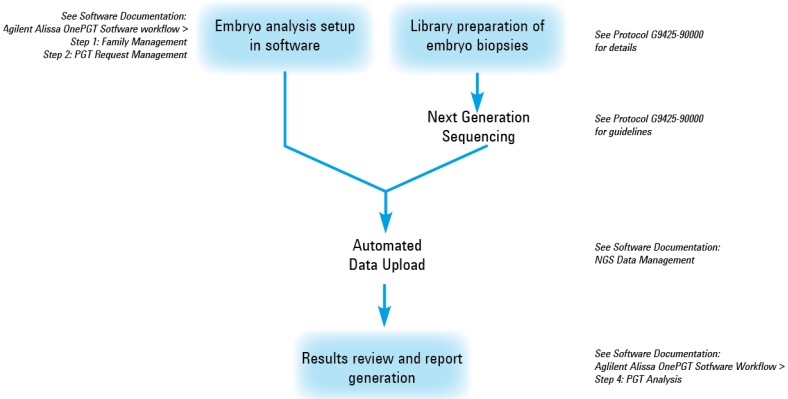
Inheritance mode	Valid reference family member setup (in addition to parents)	Limitations
Autosomal dominant	Both grandparents in affected lineage (parents of affected mother or father)	None (optimal reference setup)
	One grandparent in affected lineage (parent of affected mother or father)	Accepted but suboptimal for analysis
	Sibling	None (optimal reference setup)
Autosomal recessive	Both pairs of grandparents (both parents of carrier mother and carrier father)	None (optimal reference setup)
	Both grandparents in one carrier lineage and one grandparent in other carrier lineage	Accepted but suboptimal for analysis
	One or both grandparents in one carrier parent lineage (parents of either carrier mother or carrier father)	Only a partial conclusion on the embryo status can be made, either "Affected or Carrier" or "Carrier or Unaffected"; use of single grandparent reference is accepted but is suboptimal for analysis
	One grandparent in each carrier lineage	Accepted but suboptimal for analysis
	Sibling	None (optimal reference setup)
X-linked dominant or recessive	Both grandparents in maternal lineage	None (optimal reference setup)
	One grandparent in maternal lineage	Accepted but suboptimal for analysis
	Sibling	None (optimal reference setup)

Example PGT-M plot

An example of a haploblock-plot for a maternal chromosome is shown above. The locus of the single gene disorder is indicated by a black line or box, depending on how this locus was specified. The yellow haploblocks are in phase with the sibling, in case a sibling was used as phasing reference, or in phase with the grandfather, in case phasing was done with grandparent(s). The blue haploblocks are not in phase with the sibling, or in phase with the grandmother. For more information, refer to the software documentation section *Reviewing PGT-M Results*.

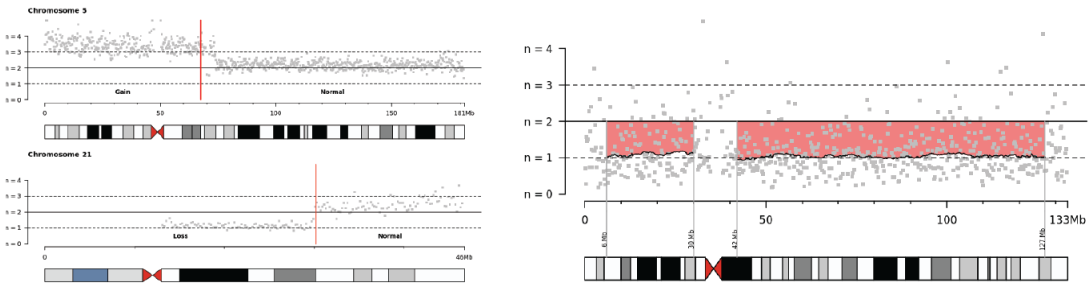
PGT-SR for translocations and PGT-A for aneuploidies

Workflow



PGT-SR and PGT-A analyses do not require the processing of gDNA from biological parents or reference family members. For more information, refer to the software documentation section *Creating a PGT Analysis*.

Example PGT-SR and PGT-A plots



An example of a PGT-SR plot (left) with an unbalanced translocation between chromosomes 5 and 21, and an example of a PGT-A plot (right) of a monosomy of chromosome 12 are shown above.

Combined PGT analysis

If desired PGT-M, PGT-SR and PGT-A analysis can be combined in a single sample analysis. For best results, we recommend at minimum including PGT-A for the chromosome carrying the monogenic disorder (for PGT-M) or for the chromosomes affected by translocation (for PGT-SR). Refer to the software documentation section *Creating a PGT Analysis* for more information on this combined analysis mode.

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